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(54) Title: DESIGN AND CONSTRUCTION OF TRANSCRIPTION TEMPLATE FOR SYNTHESIZING CELL-FREE PROTEIN, AND DILUTION BATCH-TYPE METHOD OF SYNTHESIZING CELL-FREE WHEAT GERM PROTEIN BY USING THE SAME

(54) 発明の名称: 無細胞タンパク質合成用転写鋳型の設計および構築、並びにこれを用いる希釈バッチ方式コムギ胚芽無細胞タンパク質合成法

(57) Abstract: To design and construct a transcription template for synthesizing a cell-free protein which is commonly usable, has a high translation template activity and can be easily constructed, a polynucleotide comprising a base sequence inherent to an individual vector capable of forming a complementary strand with a base sequence existing between the transcriptional terminator sequence of a marker gene (for example, a drug-tolerance gene) of the vector and Ori is provided as a 3'-terminal PCR primer, while two types of primers satisfying the requirement of not undergoing transcription from a DNA constructed by using a single primer alone, involving a polynucleotide having a sequence complementary to a base sequence containing a part of the promoter function site from the 5'-terminus of a promoter and another polynucleotide having a sequence complementary to a base sequence containing a part of the RNA polymerase recognition site from the 3'-terminus of the promoter are provided as primers for 5'-terminal PCR. Further, a method of constructing a transcription template, etc. by using them is provided.

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Title: High-throughput Cell-free Protein Expression System for Structural Proteomics

Author: Takanori Kigawa, and Shigeyuki Yokoyama

[partial translation]

[page 1015, left column, from line 14]

II. Technical development for construction of high-throughput expression system

1. Construction of flexible expression system

In cell-free system, a DNA fragment amplified by PCR can be directly used as a template for protein expression. The system successfully combined with the template preparation by PCR, enables protein preparation without cloning process from genetic resource such as cDNA library.

The authors tried to prepare a template DNA for protein expression by two-step PCR reaction in order to minimize the primer length to be prepared for respective target genes and to carry out addition of various tag sequences to the gene easily. At first, the first step PCR reaction was carried out using an inherent primer set for each DNA, and subsequently, the second step PCR was performed using a common primer set that has expression regulatory sequences (a promoter, terminator, SD sequence etc.) and a tag sequence (His-tag or GST-tag). By appropriately adjusting the reaction condition such as concentrations of respective primers, the template DNAs could be prepared efficiently. Next, the target proteins were expressed in cell-free protein synthesis system by using these templates. Because all of these reactions can be performed in a 96 or 384-well microtiter plate, it has been capable of processing a lot of samples concurrently.

Actually, randomly selected 135 mouse cDNA clones (provided by Dr. Yoshihide Hayasizaki at Genome Exploration Research Group, RIKEN) were expressed as GST-tag fusion proteins, and their synthetic amounts were shown in Fig.1. With respect to most of the proteins, more than 100 μg synthesized proteins /ml reaction mixture were obtained, and a largest protein in the selected clones, which had a molecular weight of 80kDa could be confirmed.

[page 1015, right column, from line 6]

Accordingly, it was able to construct a system for expressing and preparing a large number of proteins concurrently and rapidly to analyze the proteins. Even if all of the processes were performed manually without help of robotics, it took about only one day to require for the total process from the start of PCR to end of protein synthesis reaction. By using this system, hundreds of protein samples can be obtained in a short-term, thus, it is expected to exert a power for a study of analysis of protein molecular function such as protein-protein interaction that requires a large number of samples.

Previously, it took about one week for preparing a protein in case of using a living cell expression. Comparing the previous system, the auther's system is really a high-throughput expression system.